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## **Sex and NO — beyond regulation of vasomotor tone**

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## Editorial

## Sex and NO — beyond regulation of vasomotor tone

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**See article by Fraser et al. [43] (pages 111–118) in this issue.**

The mechanisms contributing to sex differences in susceptibility to and mortality from cardiovascular and renal disease are still unknown. Sexual dimorphisms of coronary artery disease were first suggested by Heberden in 1802 [1], and researchers have described vascular actions of estrogens since the late 19th century [2–4]. Subsequently, several studies provided indirect and direct evidence for an involvement of estrogens in the regulation of vascular tone [5–9]. Most of the mechanisms were only discovered in the past two decades (reviewed in Refs. [10,11]), following the observation by Furchgott and Zawadzki that vascular smooth muscle tone is regulated by an endothelium-derived relaxing factor [12], later identified as the free radical nitric oxide ('NO') [13]. A number of studies suggested protective effects of estrogen on atherosclerosis and related diseases [14,15], and multiple actions of estrogens on vascular homeostasis including modulation of vascular enzymes [16], vasoactive proteins and 'NO' were subsequently identified (reviewed in Refs. [10,11,17]).

'NO is a labile gas formed by multi-enzyme complexes, the 'NO synthases (NOSs) [18–20]. However, these enzymes not only catalyze the generation of 'NO from L-arginine, but also contribute to the formation of superoxide anion ('O<sub>2</sub><sup>-</sup>), which reacts with 'NO at a diffusion-limited rate to form the potent oxidant peroxynitrite (ONOO<sup>-</sup>) [21–24]. Accordingly, NOSs have been implicated in cardiovascular diseases such as hypertension, atherosclerosis, and heart failure [25]. Three major NOS isoforms have been identified [20]. Initially, NOS isoform expression was thought to be cell-/organ-specific and isoforms were termed accordingly. Meanwhile, it has been

demonstrated that 'neuronal NOS' (NOS1) [18], 'macrophage NOS' (NOS2) [19], and 'endothelial cell NOS' (NOS3) [20] or splice variants of the respective enzymes are expressed in a variety of tissues [26–29], including macrophages [19], kidney, [27,30] skeletal muscle [28], and myocardium [31,32]. The terminology formerly used to indicate calcium-dependency has also been abandoned as NOS3 also exhibits calcium-independent activity under certain conditions [33–35]. In addition, induction of 'constitutive' isoforms has been observed (i.e. induction of NOS3 by estrogen) [20,36], and the 'inducible' isoform NOS2 is constitutively expressed in certain organs (i.e. the kidney) [27,30]. Interestingly, inhibition of 'NO synthases may exert detrimental as well as beneficial effects on tissue and organ injury [25,37], which may be related to differential generation of NOS-derived free radicals [21–24].

Reperfusion injury is a complex event altering intracellular homeostasis, cardiomyocyte function, and electromechanical properties of the ischemic and post-ischemic myocardium. Increased oxidative stress as a consequence of ischemia–reperfusion is considered an important factor contributing to these abnormalities, as administration of free-radical scavengers attenuates reperfusion injury [37,38]. Calcium-independent NOS2 contributes to myocardial injury in vitro [39] and has been recently implicated in myocardial dysfunction, reperfusion injury and apoptosis in vivo [40,41]. On the other hand, NOS2 participates in the protective effects of ischemic preconditioning [31,42]. The relative contribution of NOS2-derived 'NO, 'O<sub>2</sub><sup>-</sup>, ONOO<sup>-</sup>, or nitroxyl anion ('NO<sup>-</sup>) [38] to these differential effects remains to be determined.

Using the Langendorff model, Fraser et al. now report that 17 $\beta$ -estradiol, a natural estrogen with vasodilating properties [10], increases the activity of calcium-independent but not calcium-dependent 'NO synthase in left ventricular myocardium after ischemia and reperfusion in ovariectomized rats as measured by the citrulline assay [43]. These effects were independent of NOS2 or NOS3

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protein expression and accompanied by increased myocardial cyclic guanosine monophosphate content and improved recovery of left ventricular function after ischemia as compared to untreated controls. These observations are important and point to novel modes of action by which estrogens may improve myocardial function in post-ischemic myocardium.

The findings of Fraser et al. are in contrast with previous studies reporting no effects of  $17\beta$ -estradiol on calcium-independent NOS activity, but increased calcium-dependent NOS activity as well as expression of NOS1 and NOS3 during pregnancy, a state of high estrogen levels [44–46]. Similar results were obtained using cultured endothelial cells [36,47]. One of the questions yet to be answered is whether the beneficial effects observed by Fraser et al. are due to increased activity of NOS2 or whether they also involve other NOS isoforms expressed in the myocardium [26,31,32]. Interestingly, the promoter region of the NOS2 gene contains a sequence partly resembling an estrogen responsive element [48,49] and several studies indicate that estrogen modulates NOS2 expression and/or activity. Indeed, female but not male NOS2-deficient mice are more susceptible to death due to endotoxemia than their wild-type counterparts, suggesting that estrogen modulates NOS2-mediated protection [50]. Furthermore, it has been demonstrated that  $17\beta$ -estradiol increases expression of NOS2 through an estrogen-receptor mediated mechanism in rat aortic vascular smooth muscle [49]. In contrast,  $17\beta$ -estradiol treatment suppresses the expression of NOS2 but elevates endothelial NOS3 expression in transplanted arteries [51], suggesting different mechanisms of action of  $17\beta$ -estradiol in different disease states.

Another unanswered question is whether the improvement of myocardial function after  $17\beta$ -estradiol therapy is related to the actions of 'NO itself such as vasodilation [13], improvement of myocardial perfusion [32], and increased myocardial contractility [52], or whether the beneficial effects are secondary to other mechanisms. As indicated by the authors, increases of myocardial cyclic guanosine monophosphate content may not solely derive from NOS, since other intracellular sources of intracellular cyclic guanosine monophosphate exist. Increases of cGMP may be due to scavenging of  $\text{O}_2^-$  by  $17\beta$ -estradiol, which is a potent antioxidant [10,53]. Moreover, 'NO and/or estrogen may directly interfere with mechanisms downstream of 'NO generation such as apoptosis [54–57] or protein kinase C activation [37,58], which contribute to ventricular contractility, have been implicated in reperfusion injury and modulation of which may attenuate post-ischemic myocardial injury [57,59].

The observations made by Fraser et al. may have clinical implications. Estrogen replacement therapy in postmenopausal women is associated with a reduction in cardiovascular mortality [11] and increased levels of plasma nitrate/nitrite, an indirect marker of 'NO pro-

duction [60]. Whether, and by what mechanisms, estrogen replacement therapy also contributes to myocardial protection in postmenopausal women as suggested by the initial observation that women have less angina pectoris than similarly aged men made by Heberden 200 years ago [1] and whether these effects are antagonized by certain progestogens [53,61] requires further study.

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